IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : 10/520,470
Applicant : Thomas TUSCHL et al
Filed : January 7, 2005
TC/A.U. : 1635
Examiner : Dana H. Shin

Docket No. : 2923-673
Customer No. : 6449
Confirmation No. : 5503

RESPONSE

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

October 7, 2008

Dear Sir:

In the Office Action dated May 7, 2008, claims 1, 3-9, 11-16, 20, 32-36 and 38-41, in the above-identified U.S. patent application were rejected. Reconsideration of the rejections is respectfully requested in view of the following remarks. Claims 1, 3-9, 11-16, 20, 32-36 and 38-41 remain in this application, claims 2, 10, 17-19, 21 and 37 have been canceled, and claims 22-31 have been withdrawn.

In the only remaining rejection, claims 1, 3-9, 11-16, 20, 32-36, and 38-41 were rejected under 35 USC §103(a) as unpatentable over Tijsterman in view of Elbashir and McSwiggen. Applicants previously pointed out that Tijsterman's *C. elegans* model was believed to have a different mechanism of action than mammals as there was no evidence of RISC activity in *C. elegans*. Tijsterman discloses only post transcriptional gene silencing in *C. elegans* which was not expected to be predictive of results in mammalian cells in vitro or in vivo. *C. elegans* and plants are special in their RNAi mechanisms as they require RNA-dependent RNA polymerase (RdRP) genes which are involved in amplifying the

trigger dsRNA, for this process. These genes are absent from *Drosophila* and mammalian cells. In addition in *Drosophila* and mammalian cells, RNAi is of transient nature and restricted to cells that receive an RNAi trigger. In *C. elegans* and plants, silencing signals can spread throughout the entire organism. In view of these differences in the biology and mechanism of RNAi among different species, one skilled in the art would not predict, based on studies in *C. elegans* that silencing may also be detectable in mammalian cell systems. Tuschl, Zamore, Lehmann, Sharp, and Bartel, Genes & Development, 1999, showed that long single-stranded RNA, in contrast to long dsRNA, was not a trigger of RNAi in *Drosophila* cell lysates. In view of Tijsterman's disclosure, one could speculate that the introduction of the antisense molecule led first to RdRP-dependent dsRNA synthesis, an event that would be absent in mammals. Thereafter, regular RNAi would follow. The fact that antisense molecules trigger RNAi in rde-1 and 4 mutants would be consistent with this as these genes are required for long dsRNA triggered RNAi and RdRP mutants were never tested. This is supported by the disclosure in Tijsterman which states:

> "So where do these asRNAs fit in the RNAi pathway? One possibility is that the first step of RNAi (that is, long dsRNA diced into siRNAs) is bypassed by the administration of antisense oligomers. This predicts that asRNAs will also bypass the requirement for DCR-1, the protein that fulfills this function in RNAi (1 2 , 18-20), However, we failed to observe silencing of germline-expressed GFP in dcr-1 animals that were injected with GFP asRNAs (Fig. 1F). Furthermore, if the asRNAs were to function as siRNAs, they should be incorporated in a multicomponent nuclease, designated RISC (3), that degrades homologous mRNAs. Recently, using a Drosophila cell-free system, it was found that synthetic ds-siRNAs lead to RISC (21) but only if they are of the right size: 20 to 23 nt but not longer (4, 22). We found that 15- and 18-nt asRNAs were ineffective; but asRNAs of 22 nt and longer, up to 40 nt, were fully active to trigger gene silencing in C. elegans (Fig. 1E), suggesting that these asRNA molecules are taking another route to silence gene expression.
>
> An alternative explanation is that asRNAs prime RNA synthesis

> on the mRNA, thus resulting in dsRNA that might then be a substrate for DICER-dependent degradation. This would explain why a broad range of asRNAs (22 to 40 nt long) is proficient in triggering gene silencing and why efficient gene silencing depends on the temporal coexistence of substrate and target. In favor of this idea, we observed that modifying the 3' end of the asRNAs to prohibit polymerase action reduces the efficiency of gene silencing severely (Fig. 1E). The helicase activity of MUT-14 might thus act to permit de novo RNA synthesis on the target. Indeed, putative RNA-dependent RNA polymerases (RdRPs) are involved in RNAi and posttranscriptional gene silencing (PTGS) (23-26). In addition, biochemical and genetic support for RdRP action in amplifying the RNAi response was recently obtained (11, 27). In C. elegans, ego-1 is required for RNAi of germ line-expressed genes (23); unfortunately, we could not address the role of EGO-1 directly because of sterility and strongly disrupted gonads in ego-1 mutants."

In view of the differences between nematode/plant and fly/mammals, one would not expect Tijsterman's disclosure to be predictive of results in mammalian cells. This is further supported by other publications such as:

1. Mol Cell. 2002 Sep;10(3):537-48, which provides evidence that siRNAs function as guides, not primers, in the Drosophila and human RNAi pathways.

2. Schwarz DS, Hutvágner G, Haley B, Zamore PD, which indicates that in Drosophila, two features of small interfering RNA (siRNA) structure--5' phosphates and 3' hydroxyls--are essential for RNA interference (RNAi). As in Drosophila, a 5' phosphate is required for siRNA function in human HeLa cells. In contrast, no evidence was found in flies or humans for a role in RNAi for the siRNA 3' hydroxyl group. In vitro data suggests that in both flies and mammals, each siRNA guides endonucleolytic cleavage of the target RNA at a single site. The underlying mechanism of RNAi is conserved between flies and mammals and RNA-dependent RNA polymerases are not required for RNAi in these organisms.

Elbashir (Nature 2001, 411:494-498) does not cure the deficiencies in Tijsterman as Elbashir does not use single stranded RNA and does not suggest or disclose a distribution of RNAi beyond the cellular walls either. In later transfection experiments of cell cultures, only the transfected cells not the untransfected cells showed a knock-down, although the untransfected cells were directly located beside the transfected cells. This is clearly different from *C. elegans* and plants where silencing signals can spread throughout the entire organism. In addition, Elbashir used duplexes of 21-nucleotide RNAs to mediate RNA interference in cultured mammalian cells not single stranded RNA. In view of these differences, one skilled in the art would not expect Tijsterman's *C. elegans* results to be predictive of results in mammals in view of Elbashir. McSwiggen was cited for the disclosure of chemical modifications for increased stability. McSwiggen does not suggest or disclose that single stranded RNA can be used to mediate RNA interference in mammalian cells and thus does not cure the deficiencies in Tijsterman and Elbashir. Applicants contend that due to the unpredictability in the art at the time the present invention was made, the combination of Tijstermann, Elbashir and McSwiggen does not suggest that single stranded RNA can be used to mediate RNA interference in mammalian cells. At the time the present invention was made, it was evident that in *Drosophila* systems (e.g. *Drosophila* lysates) there is an active single-strand nuclease which immediately degrades single-stranded RNA if it is not protected against nuclease with a GpppG cap structure. Short 5' phosphorylated RNA would be immediately degraded. As discussed above, the underlying mechanism of RNAi is conserved between flies and mammals. Surprisingly, the present inventors found

that lysates from mammalian cells are different and that single-stranded RNAi is possible. In addition, based on the literature available at the priority date of the present application, one skilled in the art would have concluded that it would have been hopeless to use short single-stranded RNA molecules for RNAi in mammalian systems since the mechanism of amplification as shown in Tijsterman is lacking. Applicants respectfully contend that one skilled in the art would have known that *C. elegans* and mammals have different mechanisms by which RNAi is initiated and would not have expected RNA molecules which work in *C. elegans* to be predictive of results in mammals. In view of the above discussion, applicants request that this rejection be withdrawn.

Enclosed with this response is a declaration signed by Dr. Thomas Tuschl which discusses the literature cited above.

Applicants respectfully submit that all of claims 1, 3-9, 11-16, 20, 32-36, and 38-41 are now in condition for allowance. If it is believed that the application is not in condition for allowance, it is respectfully requested that the undersigned attorney be contacted at the telephone number below.

In the event this paper is not considered to be timely filed, the Applicant respectfully petitions for an appropriate extension of time. Any fee for such an extension together with any additional fees that may be due with respect to this paper, may be charged to Counsel's Deposit Account No. 02-2135.

Respectfully submitted,

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